# **Coupling between tolerance and resistance differs between related *Eimeria* parasite species: implications for co-evolution with their mouse hosts**

# Abstract

Resistance (the host’s capacity to reduce parasite burden) and tolerance (the host’s capacity to reduce impact on its health for a given parasite burden) manifest two different lines of defenses. Theoretical models predict genetically fixed levels of tolerance, if uncoupled from resistance. Empirical observations, however, suggest coupling between resistance and tolerance. Either high resistance is leading to low tolerance in a trade-off between the two or the two are positively correlated because of redundancy in underlying (immune) processes. We here tested whether different parasite species could show difference in this coupling between tolerance and resistance.

We tested this in infections with two closely related parasite species of genus *Eimeria.* We measured proxies for resistance ((inverse of) number of parasite transmission stages (oocysts) per gram of feces at the day of maximal shedding) and tolerance (slope per host strain of maximum relative weight loss compared to day of infection on number of oocysts per gram of feces at the day of maximal shedding) in four inbred mouse strains belonging to two mouse subspecies, *Mus musculus domesticus* and *M. m. musculus*.

We found a negative correlation between resistance and tolerance against *E. falciformis*, while the two are uncoupled against *E. ferrisi*. The first parasite species can either be tolerated or resisted, not both, the latter is tolerated and resisted rather independently by different mouse genotypes.

This might be explained by intrinsic parasite components, e.g. length of life cycle and replication rate. We argue that host parameters can be studied largely irrespective of parasite strains if coupling is absent (*E. ferrisi*) but host-parasite co-evolution is best studied in a system with coupled tolerance and resistance (*E. falciformis*).

# Introduction

Parasites are ubiquitous in natural systems, causing damages to their hosts and interacting closely with them over generations. Parasites are the main selective force in the evolution of host defenses and the immune system [(Schmid-Hempel, 2009)](https://www.zotero.org/google-docs/?o5QpeJ).

Traditionally, a large number of studies only focused on parasites counts, an approach that does not allow to draw satisfactory conclusions on the host fitness, i.e. its ability to pass on its genes to the following generation (Råberg, Graham, & Read, 2009; Kutzer & Armitage, 2016). For example, hybrids of the house mouse subspecies *Mus musculus musculus* and *M. m. domesticus* show reduced parasite load compared to both parental subspecies (Baird et al., 2012; Balard et al., 2019). Interpretations of these results in terms of health or even fitness effects have been attempted (Sage, Heyneman, Lim, & Wilson, 1986) and criticised (Baird & Goüy de Bellocq, 2019).

Host defense mechanisms evolve to alleviate the detrimental effect of parasites. They can be categorised into two components: resistance and tolerance (Little, Shuker, Colegrave, Day, & Graham, 2010). Resistance is the ability of a host to reduce parasite burden. Resistance thus has a negative effect on parasite fitness and can lead to antagonistic co-evolution. It results from defense against parasite infection or proliferation early after infection (Råberg et al., 2009). In so called “matching allele models”, fluctuating host and parasite genotypes arise, and balancing selection maintains resistance alleles polymorphic (ref). Resistance has been the classical “catch all” measure for host-parasite systems, but recently it has been shown to be incomplete, especially with respect to potential fitness effects on the host (ref).

Tolerance of the host does not alter the fitness of the parasite and thus does not lead to antagonistic co-evolution. Tolerance alleles are thus predicted by theoretical models to evolve to fixation (Roy & Kirchner 2000; Miller et al. 2005). From a mechanistic perspective tolerance alleviates damage caused by parasites. Excessive immune response underlying resistance against parasites can itself result in immunopathology (Graham, Allen, & Read, 2005) and tolerance reduces this negative effect (Medzhitov, Schneider, & Soares, 2012). The host uses stress response, damage repair and cellular regeneration as tolerance mechanisms (Soares, Teixeira, & Moita, 2017). Effects on the parasite can be seen as physiologically independent from tolerance in each infected individual and can evolve independently in a population. This predicts a decoupling of tolerance and resistance at both the individual level in the physiological situation and at population level over evolutionary time.

Resistance and tolerance are often found to be negatively correlated in empirical studies. Inbred laboratory mouse strains lose weight upon infection with *Plasmodium chabaudi.* The extent of this impact on host health is negatively correlated with the peak number of parasites found in the blood (Råberg, Sim, & Read, 2007), meaning that mouse strains with higher resistance present lower tolerance. Similarly, infections of sea trout (Salmo trutta trutta) and Atlantic salmon (Salmo salar) with the trematode Diplostomum pseudospathaceum showed that resistance and tolerance were negatively correlated when assessing mean levels of both traits in different host populations (Klemme & Karvonen, 2016). The a balance between costs associated with parasitism, with resistance and with tolerance might determine the optimal level of both defense mechanisms (Sheldon & Verhulst, 1996). Overall, this work suggests trade-offs between resistance and tolerance.

Resistance and tolerance can also be positively correlated. Chemical defense and enhanced (re-)growth of tissue involve the same metabolic pathwayin the model plant *Arabidopsis thaliana.* Resistance mediated by a chemical compound and tissue regeneration, as a mechanism for tolerance, are thus simultaneously induced by herbivory (Mesa, Scholes, Juvik, & Paige, 2017). Genetic association studies of resistance and tolerance of *Drosophila melanogaster* against the bacterium *Providencia rettgeri* have shown genetically correlated effects, meaning that changes of two traits in the same direction associate with most loci (Howick & Lazzaro, 2017). Finally, selection on tolerance against parasitic helminths has been shown in an isolated population of Soay Sheep. While correlation of resistance and tolerance was not detected in this work, polymorphism and ongoing selection of tolerance need an explanation likely found in a link between resistance and tolerance mechanisms (Hayward et al 2014). Redundancy of resistance and tolerance can mean they are either physiologically or genetically linked, controlled by the same metabolic pathways or genetic architecture and hence positively correlated.

(Restif & Koella, 2004; Fornoni, Nuñez-Farfan, Valverde, & Rausher, 2004).

The studies cited above used different methodological frameworks. Depending on this they find either no (theoretical models and field studies), negative (comparison between populations in experimental infections) or positive (comparisons between individuals and field studies) coupling of resistance and tolerance.

We here hypothesize that coupling between resistance and tolerance (or absence thereof) depends not only on host factors, but could also be conditioned by parasite intrinsic factors. We tested differences in the resistance-tolerance coupling upon infection with two closely related parasite species. We infected four inbred mouse strains representative of two house mouse subspecies, *M. m. domesticus* and *M. m. musculus*, with three parasite isolates representative of two naturally occuring parasite species, the protozoan parasite *Eimeria ferrisi* and *E. falciformis* (Jarquín-Díaz, Balard, Jost, et al. 2019). *Eimeria*spp. are monoxenous parasites that expand asexually and reproduce sexually in intestinal epithelial cells, leading to malabsorption of nutrients, tissue damage and weight loss (Chapman et al., 2013). The evolutionary history of these different *Eimeria* species in the two house mouse subspecies is unknown and it is unclear whether subspecies-specific adaptation exists in one or the other. We tested (1) if coupling between resistance and tolerance of each host differs between both parasite species; and (2) local adaptation of *E. ferrisi* using a parasite isolated in a *M. m. domesticus* host and one in a *M. m. musculus* host. We expected that local (host sub-species) adaptation would be more likely if resistance and tolerance are coupled for this parasite.

# Material and methods

## Parasite isolates

The three parasite isolates used in this study were isolated from feces of three different *M. m. domesticus/M. m. musculus* hybrid mice captured in Brandenburg, Germany, in 2016 (capture permit No. 2347/35/2014). Hybrid index (HI) of each individual wild-caught mouse was calculated to account for the admixture of mouse genomes as a proportion of *M. m. musculus* alleles in a set of 14 diagnostic markers (Balard et al., 2019). The parasite isolates belong to both the most prevalent *Eimeria* species in this area, namely *E. ferrisi* (isolates Brandenburg64 and Brandenburg139) and *E. falciformis* (isolate Brandenburg88)(Jarquín-Díaz et al., 2019). Isolate Brandenburg64 was isolated in a 92% *M. m. domesticus* individual (HI=0.08), isolate Brandenburg139 in a 85% *M. m. musculus* (HI=0.85) and isolate Brandenburg88 in a 80% *M. m. domesticus* (HI=0.2). Pre-patency and the peak day of parasite shedding for these isolates were estimated during infection in NMRI laboratory mice (Al-khlifeh et al., 2019) which were also used for serial passaging of all the isolates. Parasite infective forms (oocysts) were recovered by flotation in saturated NaCl solution followed by washing and observation under light microscope (following the protocole described in Clerc, Fenton, Babayan, & Pedersen, 2019) and stored at room temperature in 1mL of 2% potassium dichromate for a maximum of 2 months before infection of the wild-derived mouse strains. Oocysts were allowed to sporulate 10 days before infection in a water bath at 30°C.

## Mouse strains

We used four wild-derived inbred mouse strains: two representing *M. m. domesticus*: **SCHUNT** (Locality: Schweben, Hessen, Germany [N: 50° 26’, E: 9° 36’] (Martincová, Ďureje, Kreisinger, Macholán, & Piálek, 2019)) and **STRA** (Locality: Straas, Bavaria, Germany [N: 50° 11’, E: 11° 46’] (Piálek et al., 2008), and two derived from *M. m. musculus*: **BUSNA** (Locality: Buškovice, Bohemia, Czech Republic [N: 50° 14’, E: 13° 22’] (Piálek et al., 2008)) and **PWD** (Locality: Kunratice, Bohemia, Czech Republic [N: 50° 01’, E: 14° 29’] (Gregorová & Forejt, 2000))(**Figure 1**). Age of the mice at the time of infection ranged between 7.6 and 21.4 weeks. All mouse strains were maintained before infection in the Institute of Vertebrate Biology in Studenec (licence number 61974/2017‐MZE‐17214; for further details on strains see <https://housemice.cz/en>).

Parasites of the *Eimeria* genus are known to induce host immune protection against reinfection (Rose, Hesketh, & Wakelin, 1992; Smith & Hayday, 2000). To ensure that our mice were *Eimeria*-naive, mice fecal samples were tested before infection for the presence of *Eimeria* spp. oocysts, by flotation in saturated NaCl solution followed by washing and observation under light microscope.

## Experimental infection

Mice were kept in individual cages during infection. Water and food (SNIFF, Rat/Mouse maintenance feed 10 mm) were provided *ad libitum* supplemented with 1 g of sunflower and barley seeds per day. Mice were orally infected with 150 sporulated oocysts of one *Eimeria* isolate suspended in 100 µl phosphate-buffer saline (PBS) and monitored daily until their sacrifice by cervical dislocation at 11 days after infection (dpi) (experiment license Reg. 0431/17). Individuals presenting severe health deficiency and/or a weight loss approaching 18% relative to their starting weight were sacrificed earlier. Weight was recorded and feces collected on a daily basis. Fecal pellets were collected every day from each individual cage and suspended in 2% potassium dichromate. Parasite oocysts were recovered using NaCl flotation (see above).

All individuals were negative for *Eimeria* at the beginning of our experiment (before infection of first batch, as described in the next paragraph). In total, 108 mice were infected. Mice were randomly allocated to experimental groups ensuring homogeneous distribution of ages and sexes between groups. Our experiments were conducted in four (partially overlapping) consecutive batches for logistical reasons. The first two batches were infected with the two *E. ferrisi* isolates (Brandenburg64 and Brandenburg139), the third and forth by one *E. ferrisi* isolate (Brandenburg64) and one *E. falciformis* isolate (Brandenburg88). Our experimental design is summarized in **Table 1,** (chronology of experimental batches can be scrutinized in supplementary table XYZ).

We observed *Eimeria* oocysts in the feces of 9 mice belonging to the last experimental batch at the day of infection, likely due to cross-contamination between batches. Moreover, before arrival to the infection facility, nematode eggs were observed in flotated feces of mice belonging to all genotypes. Nematode infection is common in breeding facilities (Baker, 1998). Despite treatment of the first infection batch of mice (22 mice) with anthelminthics (Profender®, Bayer AG, Levekusen, Germany) following the protocole of Mehlhorn et al. (2005), nematodes were still detected with PCR (following the protocole of Floyd, Rogers, Lambshead, & Smith, 2005) in randomly sampled fecal samples a week later. We therefore decided not to treat mice of the following infection batches. For following statistical tests, we considered the full data set and a conservative data set in which cross-contaminated animals and animals treated by anthelminthic are removed.

## Statistical analyses

### Modeling of resistance, impact of parasite on host and tolerance

As resistance is the capacity of a host to reduce its parasite burden, it is usually estimated by the inverse of infection intensity (Råberg et al., 2009). Pre-patency (the time to shedding of infectious stages, so called oocysts) is longer for *E. falciformis* (7 days) than for *E. ferrisi* (5 days) (Al-khlifeh et al., 2019). Therefore, as a proxy of resistance we used the (inverse of) number of oocysts per gram of feces (OPG) at the day of maximal shedding. We found this measurement to be tightly correlated with the sum of oocysts shed throughout the experiment (Pearson correlation coefficient 0.91). Due to the aggregation characteristic of parasites (Shaw & Dobson, [1995](https://onlinelibrary.wiley.com/doi/full/10.1111/jeb.13578" \l "jeb13578-bib-0070)), the appropriate distribution for maximum number of OPG was found to be the negative binomial distribution. This was confirmed based on log likelihood, AIC criteria and goodness-of-fits plots (density, CDF, Q-Q, P-P plots) (R packages MASS (Venables & Ripley, 2002) and fitdistrplus (Delignette-Muller & Dutang, 2015)).

Both parasite species provoke inflammation, cellular infiltration, enteric lesions, diarrhea, and ultimately weight loss (Ankrom, Chobotar, & Ernst, 1975; Ehret, Spork, Dieterich, Lucius, & Heitlinger 2017; Schito et al., 1996; Al-khlifeh et al., 2019). Therefore, the impact of parasites on host health was measured as the maximum relative weight loss compared to day 0 (body weight measured at the start of the experimental infection). Tolerance is usually defined as a reaction norm, i.e. the regression slope of host fitness (approximated by health condition) on infection intensity per genotype (Råberg et al., 2009). Therefore tolerance was assessed as the slope of maximum relative weight loss compared to day 0 on number of OPG at the day of maximal shedding, within each mouse strain and for each parasite isolate.

### Statistical design

Maximum OPG (model 1) and relative weight loss (model 2) were modelled separately as a response of mouse strain (N=4), parasite isolate (N=3) and their interaction, using a negative binomial generalised linear model for maximum OPG, and a linear model for relative weight loss. For tolerance (model 3), we performed a linear regression with null intercept (as each mouse was controlled against itself at start of the experiment, before losing weight or shedding parasite), modelling relative weight loss as a response of maximum OPG interacting with mouse strain (N=4), parasite isolate (N=3) and their interaction. To test the significance of the marginal contribution to each parameter to the full model, each parameter was removed from the full model, and the difference between full model and sub-model was assessed using likelihood ratio tests (G).

For each of our three models, if the response differed between parasite isolates (i.e. if the variable “parasite isolate” was significant), we asked within each infection group if the response differed between mouse genotypes (i.e. variable “mouse strain” significant) using likelihood ratio tests (G) as described above. Eventually, if this was the case, post-hoc multiple comparison tests (Tukey Multiple Comparisons of Means) were performed to test the significant difference in response of each host against all others (R package emmeans (Lenth, 2019)).

We verified for each analysis the absence of impact of both previous contamination by *Eimeria* and anthelminthic treatment on our results on a conservative data set excluding the 22 mice treated by anthelminthics and the 9 mice showing contaminant infections. All analyses were performed using the R software version 3.5.2 (R Development Core Team, 2018). Graphics were produced using the R package ggplot2 (Wickham, 2016) and compiled using the free software inkscape ([https://inkscape.org](https://inkscape.org/)). All codes and data used for this article can be found at: <https://github.com/alicebalard/Article_RelatedParasitesResTol>

# Results

## 1. General parasitology

The life cycle of all isolates was successfully completed in all mouse strains (**Figure 2**). For *E. ferrisi* (both isolates), the pre-patent period was 5 dpi and the median day of maximal oocyst shedding was 6 dpi (standard deviation sd=0.73 and 0.61, respectively). The median day of maximum weight loss was 5 dpi for both isolates (sd=2.1 and 1.9 respectively). For *E. falciformis* (isolate Brandenburg88) pre-patency was 7 dpi, median day of maximal shedding was 8 dpi (sd=1.2) and median day of maximal weight loss 9 dpi (sd=1.5). All tested *Eimeria* isolates infected all individuals of the tested mouse strains.

A considerable number of *M. m. musculus* mice (8/14; 5 of BUSNA and 3 of PWD) infected with *E. falciformis* (isolate Brandenburg88) died (or had to be sacrificed at humane end points specified in animal experimental procedures) before the peak of oocyst shedding. Moreover, one *M. m. domesticus* mouse (strain SCHUNT) infected by *E. ferrisi* isolate Brandenburg139 had liquid diarrhea in the peak shedding day, making its feces not collectable. These mice were considered missing data.

## **2. Resistance to *Eimeria spp.* in different mouse strains**

To test differences of resistance between mouse strains infected by each parasite isolate, we modelled the maximum number of OPG as a measure of (inverse of) resistance (**Figure 3A**). Considering the 99 mice alive by the time of median shedding peak of each parasite isolate, we found statistically significant effects of parasite isolate (LRT: G=35.5, df=8, P<0.001), mouse strain (LRT: G=36.3, df=9, P<0.001) as well as an interaction between parasite isolate and mouse strain (LRT: G=21.8, df=6, P<0.01). This means that mouse strains are differently resistant depending on parasite isolates.

We then modelled the maximum number of OPG as a measure of (inverse of) resistance within our three infection groups, and found mouse strain significant is mice infected by *E. ferrisi* Brandenburg64 (LRT: G=19, df=3, P<0.001) and *E. falciformis* Brandenburg88 (LRT: G=11.6, df=6, P<0.01). For these two infection groups, we performed post-hoc multiple comparison tests.

Upon infection with *E. ferrisi* Brandenburg64, the SCHUNT M*. m. domesticus* strain was more resistant than both *M. m. musculus* strains (Tukey test: SCHUNT-BUSNA: P<0.01; SCHUNT-PWD: P<0.001; predicted average million OPG shed at peak and 95%CI: SCHUNT (*M. m. domesticus*): 0.5 [0.3, 0.6]; STRA (*M. m. domesticus*): 0.8 [0.6, 1.2]; BUSNA (*M. m. musculus*): 1.1 [0.8, 1.6]; PWD (*M. m. musculus*): 1.6 [1.1, 2.4]). Upon infection with *E. ferrisi* Brandenburg139, all mouse strains were found equally resistant (predicted average million OPG shed at peak and 95%CI: SCHUNT: 0.5 [0.3, 0.8]; STRA: 0.6 [0.4, 1.1]; BUSNA: 0.5 [0.3, 0.8]; PWD: 0.9 [0.5, 1.5]).

Upon infection with *E. falciformis* Brandenburg88 the PWD *M. m. musculus* strain was found more resistant than the STRA *M. m. domesticus* strain (Tukey test: STRA-PWD: P<0.001; predicted average million OPG shed at peak and 95%CI: SCHUNT (*M. m. domesticus*): 1.1 [0.7, 1.9]; STRA (*M. m. domesticus*): 2.1 [1.3, 3.4]; BUSNA (*M. m. musculus*): 1.4 [0.5, 3.5]; PWD (*M. m. musculus*): 0.4 [0.2, 0.8]). The second strain of *M. m. musculus,* BUSNA, was represented by only 2 animals, as 5 had died before the peak of shedding, having shed few or no oocysts.

In summary, we found different resistance between mouse strains infected by *E. ferrisi* Brandenburg64 and *E. falciformis* Brandenburg88, but not *E. ferrisi* Brandenburg139.

## **3. Impact on weight of *Eimeria spp.* in different mouse strains**

We then modelled the weight loss upon infection relative to day 0 as a proxy for impact on host health of the full data set (N=108) in response to mouse strain, parasite isolate, and their interaction (**Figure 3B**). We found statistically significant differences between parasite isolates (LRT: G=47.6, df=8, P<0.001), mouse strains (LRT: G=38, df=9, P<0.001) and their interaction (LRT: G=16.2, df=6, P=0.01). In infections with parasite isolates *E. ferrisi* Brandenburg64 (LRT: G=14.6, df=3, P<0.01) and *E. falciformis* Brandenburg88 (LRT: G=18.3, df=3, P<0.001) relative weight loss differs significantly for different mouse strains. For these two infection groups, we performed post-hoc multiple comparison tests.

Upon infection with *E. ferrisi* Brandenburg139, all mouse strains were affected equally, losing 6 to 10% of their initial weight at maximum (predicted average relative weight loss and 95%CI: SCHUNT (*M. m. domesticus*): 8% [4% – 12%]; STRA (*M. m. domesticus*): 7% [3% – 11%]; BUSNA (*M. m. musculus*): 6% [2% – 10%]; PWD (*M. m. musculus*): 8% [4% - 12%]). When infected with the second *E. ferrisi* isolate (Brandenburg64), one *M. m. musculus* strain (PWD) lost more weight than both *M. m. domesticus* strains (Tukey test: PWD-SCHUNT: P=0.03, PWD-STRA: P<0.01; predicted relative weight loss and 95%CI: SCHUNT (*M. m. domesticus*): 5% [2% – 7%]; STRA (*M. m. domesticus*): 3% [1% – 6%]; BUSNA (*M. m. musculus*): 7% [4% – 10%]; PWD (*M. m. musculus*): 9% [6% - 12%]).

The differences in relative weight loss were found more pronounced between strains upon infection with *E. falciformis* isolate (Brandenburg88), with one *M. m. domesticus* strain (STRA) less affected by the infection than both *M. m. musculus* strains (Tukey test: STRA-BUSNA: P<0.01; STRA-PWD: P<0.01; predicted average relative weight loss and 95%CI: SCHUNT (*M. m. domesticus*): 10% [6% – 15%]; STRA (*M. m. domesticus*): 6% [2% – 10%]; BUSNA (*M. m. musculus*): 18% [14% – 21%]; PWD (*M. m. musculus*): 19% [15% - 23%]). Of note, after losing weight, an important number of *M. m. musculus* died of infection by *E. falciformis* (3 out of 7 PWD and 5 out of 7 BUSNA). Such mortality was not found in *E. ferrisi* infected animals.

Eventually, when comparing the above values of relative weight loss of each mouse strain across infection isolates, both *M. m. domesticus* strains (STRA and SCHUNT) lost on average between 3 and 10% of their starting weight for all infections. Both *M. m. musculus* strains (BUSNA and PWD), in contrast, were more affected by *E. falciformis* (18-19% relative weight loss, and high mortality as described above) than by *E. ferrisi* isolates (6 to 9% relative wight loss). These are indications than *M. m. musculus* are more affected by *E. falciformis* than by *E. ferrisi*, while *M. m. domesticus* do not show such heterogeneity.

## **4. Tolerance to *Eimeria spp.* in different mouse strains**

To combine the tow measurements analysed previously into a tolerance estimate, we modelled the weight loss upon infection relative to day 0 as a linear regression of maximum OPG. We allowed slopes of this regression to differ for *Eimeria* isolate and mouse strain and for all combinations between two. on the full data set excluding mice that died before the infection peak (N=99). We found statistically significant differences of the tolerance slope between parasite isolates (LRT: G=30.2, df=8, P<0.001), mouse strains (LRT: G=30.6, df=9, P<0.001) and their interaction (LRT: G=24, df=6, P<0.001)(**Figure 4**).

We found no difference of tolerance between mouse strains for both *E. ferrisi* isolates (relative average weight loss in % per million OPG and 95%CI: Brandenburg139: SCHUNT (*M. m. domesticus*): 12 [5-29], STRA (*M. m. domesticus*): 11 [4-19], BUSNA (*M. m. musculus*): 10 [1-18]; PWD (*M. m. musculus*): 7 [3-13]; Brandenburg64: SCHUNT (*M. m. domesticus*): 6 [0-12], STRA (*M. m. domesticus*): 3 [0-6]; BUSNA (*M. m. musculus*): 4 [2-6]; PWD (*M. m. musculus*): 5 [3-7]). Brandenburg64 seems better tolerated than Brandenburg139, regardless of the mouse strains.

We found different tolerance slopes between mouse strains for *E. falciformis* isolate Brandenburg88 (LRT: G=10.3, df=3, P=0.016). We performed a post-hoc multiple comparison test for this isolate, and found that PWD (*M. m. musculus*) was less tolerant than STRA (*M. m. domesticus*) (higher value of the slope of relative weight loss per OPG; Tukey test: P=0.036; relative average weight loss in % per million OPG and 95%CI: SCHUNT: 6 [2-10], STRA: 3 [0-5]; BUSNA: 9 [2-13]; PWD: 35 [22-47]). Again, due to the high mortality of the *M. m. musculus* strain BUSNA the calculated tolerance is an overestimate for this strain. In summary, we found indications than *M. m. musculus* are less tolerant to *E. falciformis* than *M. m. domesticus*, while such difference could not be found for *E. ferrisi* infections.

The conclusion of our three analyses (maximum OPG, relative weight loss, slope of the two) results were consistent with results obtained on the conservative data set (excluding anthelminthic treated and contaminated mice), thus we considered the influence of both confounding factors negligible.

# Discussion

In this study, we assessed resistance and tolerance to two closely related parasites, *E. ferrisi* (two isolates) and *E. falciformis* (one isolate), in four different inbred strains representative of two house mouse subspecies. We used this controlled infection experiment to investigate whether resistance and tolerance are correlated in this system, with two implications: from a practical “measurement” perspective we can ask whether tolerance can be predicted from the easier to measure resistance (e.g. in field sampling). In a evolutionary perspective, this might determine whether co-evolution between host and parasite can be expected, as resistance impacts parasite fitness, but tolerance can uncouple host fitness from this. If tolerance and resistance are coupled, parasite and host fitness are too.

Upon infection with the first parasite species (*E. falciformis*), we observed heterogeneity of resistance between mouse strains, one *M. m. musculus* being highly resistant, one *M. m. domesticus* lowly resistant, and the two others (one *M. m. domesticus*, one *M. m. musculus*) presenting intermediate resistance levels. Mouse strains belonging to *M. m. musculus* subspecies were far more affected (in terms of weight loss but also mortality) by this parasite than strains belonging to *M. m. domesticus* subspecies.

The more pathogenic parasite *E. falciformis* was poorly tolerated but strongly resisted by *M. m. musculus,* while *M. m. domesticus* tolerated it well but showed low levels of resistance. This might indicate trade-off between resistance and tolerance as observed in similar experimental infections for other host parasite systems before (Råberg et al., 2007; the trout study, really all we have?)

Resistance-tolerance trade off could be explained by intrinsic characteristics *E. falciformis.* This parasite has a relatively long life cycle (Al-khlifeh et al., 2019; Haberkorn, 1970), meaning that it multiplies asexually for a relative long time. If the parasite is not resisted well enough might lead to high potential tissue load and – once the parasite starts to reproduce sexually – extremely high reproductive output in strongly impacted hosts. Tissue damage is observed during sexual reproduction for this parasite (Ehret et al., 2017) and might mean that a certain level of resistance is required. But also immunopathology has been observed in advanced *E. falciformis* infections. For example, proinflammatory T cell mediators have been shown to decrease parasite load but increase body weight loss upon infection (Stange et al., 2012). This might lead to multiple different optima for resistance and tolerance.

In addition to more or less stable optima in the two mouse subspecies we could speculate two related alternative explanations. Firstly, *E. falciformis* could originally be a *M. m. domesticus* parasite dissipated into *M. m. musculus* territory by a spillover through the hybrid zone. As an argument against this explanation, no significant difference in *E. falciformis* prevalence at each side of the hybrid zone has be observed (unpublished data). Secondly, the *E. falciformis* isolate employed here was collected from a predominantly *M. m. domesticus* mouse (hybrid index 0.2). The isolate could hence be locally adapted to *M. m. domesticus*. Experiments with additional *E. falciformis* isolates (especially from *M. m. musculus*) are needed to test whether host subspecies adaptation can lead to tolerance in matching pairs of *E. falciformis* isolates and mouse subspecies. This seems plausible, as a coupling between resistance and tolerance could couple host and parasite fitness potentially leading to co-evolution. Interestingly, parasite and host co-evolution in this perspective wouldn’t be antagonistic with regards to tolerance and parasite reproduction (that is, the inverse of resistance), as co-adaptation would optimize both for host and parasite.

Upon infection by the second parasite species, *E. ferrisi*, we did not find such resistance-tolerance trade-off. One isolate presented heterogeneity of resistance, but homogeneous impact on host weight and tolerance in each mouse strain. The second parasite isolate showed uniform resistance, impact on weight and tolerance in each mouse strain. As we did not find indications of higher tolerance to Western parasite of Western host (*M. m. domesticus*) than of Eastern host (*M. m. musculus*) and vice versa, local adaptation of *E. ferrisi* is not supported. This might be explained by tolerance uncoupling parasite and host fitness and therefore making host-parasite co-evolution less likely.

*E. ferrisi* commits to sexual reproduction after a relatively short time with few cycles of asexual expansion (Al-khlifeh et al., 2019; Ankrom et al., 1975). As *E. ferrisi* infections do not reach extremely high intensities with this infection strategy, high tolerance might be the optimal strategy for both house mouse subspecies. Resistance could then evolve relatively freely without any major impact of the parasite on the hosts’ health. Enhanced virulence (reduction of host fitness upon infection e.g. due to prolonged asexual replication before commitment to sexual replication and transmission) might not evolve because the low resistance of the host already allows an optimal transmission rate, especially considering the fast production of transmission stages (Anderson & May, 1982). A global optimum of high tolerance might also be the reason why no subspecies-specific adaptation of *M. m. domesticus* or *M. m. musculus* infected strains, i.e. no increased tolerance of matching host-parasite pairs, is detected for this parasite in the present study. This is in line parasite population structure not correlated with host-structure in the house mouse hybrid zone (ref Jarquin). In summary this implies that the might expect the prevalent *E. ferrisi* to be a less likely co-evolving with it’s house mouse host than *E. falciformis*.

We found tolerance to be decoupled from resistance against *E. ferrisi,* while the two types of response against *E. falciformis* were negatively correlated, suggesting a trade-off between resistance and tolerance for this parasite. Coupling between resistance and tolerance can then differ between closely related parasite species. This finding is relevant in our system: it has been shown that hybrids between *M. m. domesticus* and *M. m. musculus* are more resistant not only to *Eimeria* but also to other parasites including pinworms (Baird et al., 2012; Balard et al., 2019) but impact on tolerance could not be measured under natural conditions (Balard et al., 2019). The effect of parasites on host fitness and in the evolution of the house mouse hybrid zone is thus still rather ambiguous. Careful distinction between parasite species is necessary when analysing the influence of host genetics on such phenotypes. We here show that it is indispensable to measure both resistance and tolerance in *Eimeria* infections of house mice, measurements that can best be made in future laboratory experiments with hybrid mice. Moreover, the contrast between resistance and tolerance coupling in two different parasite can guide research on in this host-parasite system: if the effects of hybridisation should be studied independently of potential host-parasite co-adaptation the prevalent *E. ferrisi* might be the most suitable parasite. If co-evolution between hosts and parasites should be studied the pathogenic *E. falciformis* is a more plausible target*.* In conclusion we show that the coupling between resistance and tolerance can differ between closely related parasite species and we argue that this trait of the host-parasite system determines the questions to be best approached with a particular parasite.

# Tables

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Mouse** | | **Eimeria species / isolate** | | |
| **strains** | **subspecies** | ***E. ferrisi***  **Brandenburg139** | ***E. ferrisi***  **Brandenburg64** | ***E. falciformis***  **Brandenburg88** |
| SCHUNT | *M.m.domesticus* | 7 (5M / 2F) | 14 (6M / 8F) | 6 (3M / 3F) |
| STRA | *M.m.domesticus* | 6 (2M / 4F) | 15 (8M / 7F) | 7 (4M /3F) |
| BUSNA | *M.m.musculus* | 6 (2M / 4F) | 14 (8M / 6F) | 7 (3M /4F) |
| PWD | *M.m.musculus* | 6 (3M / 3F) | 13 (10M / 3F) | 7 (1M / 6F) |

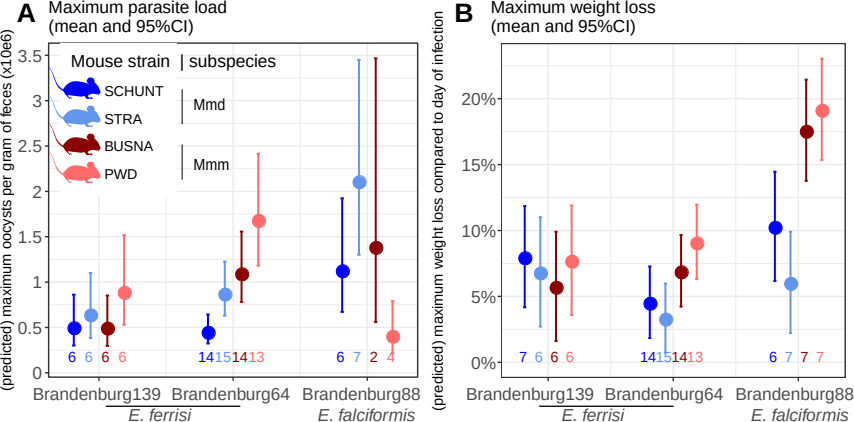
**Table 1. Infection experiment design.**

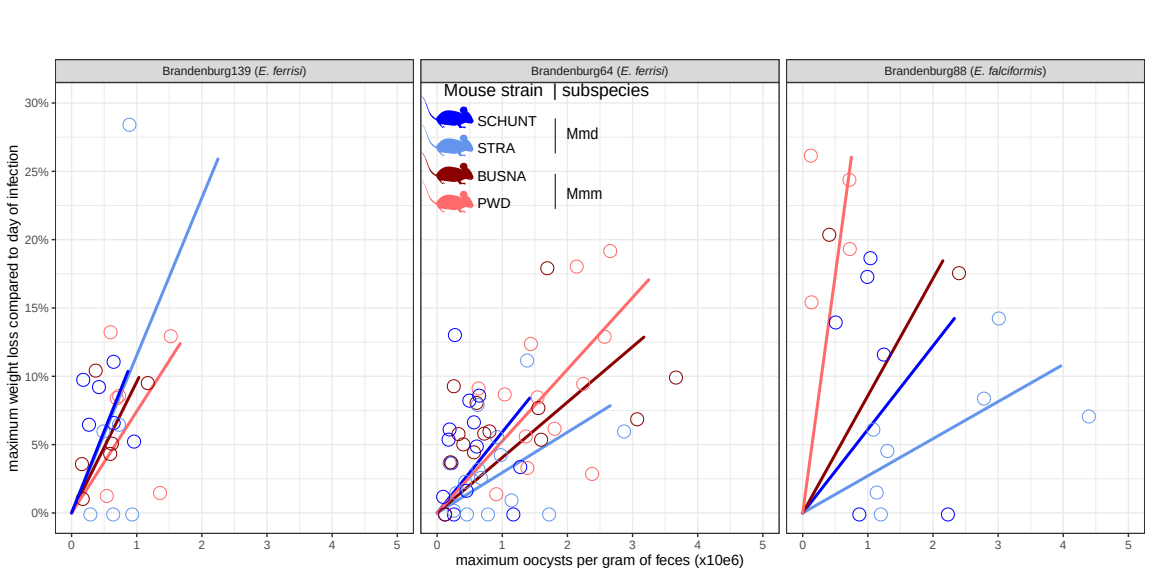
# Figures

**Figure 1. Parasite isolates and mouse strains.** The map shows locations at which mice were collected for breeding of mouse strains and isolation of parasites. The purple line is an estimation of the center of the house mouse hybrid zone between *M. m. domesticus* and *M. m. musculus* based on sampling and genotyping of mice in this area (Balard et al., 2019; Ďureje, Macholán, Baird, & Piálek, 2012, Macholán et al. 2019).



**Figure 2. Parasite density (A) and relative weight loss (B) during *Eimeria* infection.** Parasite density is calculated as number of oocysts detected (x10e6) per gram of feces, relative weight loss is calculated compared to day 0.Mean and 95% CI are plotted for each parasite isolate. All hosts strains are pooled together.

**Figure 3. Predicted maximum parasite load and maximum weight loss by mouse strain and *Eimeria* isolates.** Values under bars represent the number of animals for each group. (A) Maximum oocysts per gram of feces used as a proxy for (inverse of) resistance; (B) Impact on host health measured as the maximum weight loss during patent period relative to starting weight (%)

**Figure 4. Predicted tolerance of each mouse strain for each *Eimeria* isolates.** Tolerance is estimated by the slope of the linear regression with null intercept modelling relative weight loss as a response of maximum oocysts per gram of feces.

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